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TECHNIQUE FOR CARDIOVASCULAR MONITORING IN AWAKE
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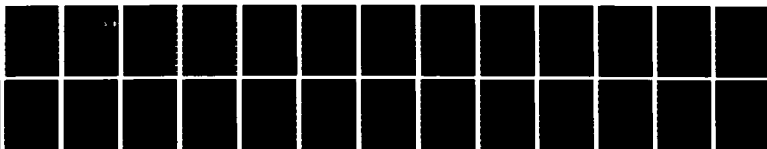
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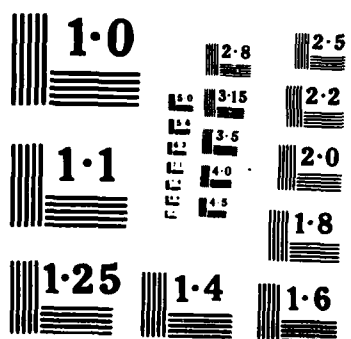
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TECHNIQUE FOR CARDIOVASCULAR MONITORING
IN AWAKE TETHERED RATS

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Running Title: Cardiovascular Monitoring in Tethered Rats

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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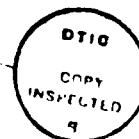
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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. A170246	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Technique for Cardiovascular Monitoring in Awake Tethered Rats		5. TYPE OF REPORT & PERIOD COVERED Interim
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Gerald W. Parker and Dale G. Martin		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS US Army Medical Research Institute of Infectious Diseases, SGRD-UIS Fort Detrick, Frederick, MD 21701-5011		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command		12. REPORT DATE 16 July 1986
		13. NUMBER OF PAGES 20 + 4 Figures
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report)
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Distribution unlimited - Approved for public release		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES To be published in Laboratory Animal Science		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) conscious F344 rat, cardiac output, arterial blood pressure, ECG		
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ABSTRACT

We have developed a tethering system for use in awake, freely moving rats that allows for repeated measurement of either cardiac output or direct arterial blood pressure and electrocardiograms (ECG). Cardiac output was measured by the thermodilution technique by using a thermocouple probe and polyethylene tubing surgically implanted in the aortic arch and superior vena cava, respectively. Arterial blood pressure and ECGs were monitored via a carotid arterial catheter and teflon-coated, stainless steel wire electrodes placed subcutaneously on the extremities. The catheters, wires, and thermocouple probe were passed subcutaneously to the dorsal cervical area and exteriorized. The animals were then attached to a rodent jacket/spring tether system and allowed to recover for at least 24 to 48 hours before experimental procedures were initiated. In 49 rats, arterial blood pressures and ECGs were analyzed. Similarly, cardiac output was measured in 27 rats. Baseline values for the model include the following: mean arterial blood pressure, 121 ± 9.5 mm Hg (mean \pm SD); heart rate, 386 ± 27 beats/min; and cardiac output, 36.4 ± 4.9 ml/min/100 g. Baseline lead II ECG showed amplitudes and intervals of 40.38 ± 0.32 msec for PR interval, 12.29 ± 2.01 msec for QRS complex, 0.879 ± 0.186 mvolts for R wave, and 0.134 ± 0.032 mvolts for T wave. In summary, this procedure facilitated repeated measurement of electrocardiographic and hemodynamic indices in awake rats; thus, comparisons can be made between baseline values and after pharmacological intervention.



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Rats are used routinely in various cardiovascular studies. However, their small size limits the amount of bioinstrumentation and thus the hemodynamic parameters that can be measured. As a result, many studies have been limited to reporting only arterial blood pressure and heart rate. With recent developments in cardiovascular monitoring techniques, it has become possible to perform more in-depth instrumentation in the rat. The purpose of this report is to describe a practical and relatively inexpensive technique for use in cardiovascular studies with the rat animal model.

In the rat, reference values for cardiac output vary from 20.7 to 51.8 ml/min/100 g (1,2) and mean arterial pressure from 90 to 150 mm Hg, depending on the strain, state of the animal, and method of measurement. Anesthetic agents have been shown to alter the stability of various hemodynamic parameters, as well as the reactivity of the cardiovascular system, to various physiological and pharmacological manipulations (3,4). Additionally, it has been shown that the stress of physical restraint results in slightly elevated blood pressures in normotensive rats (5) and hyperresponsivity to the stress in spontaneously hypertensive rats (6). Therefore, it was important for us to develop an awake model free from the influence of anesthesia and physical restraint. Although the rat has been used extensively in cardiovascular research, few have reported the simultaneous and repeated measurement of ECG and arterial blood pressure in the awake, freely-moving animal. Likewise, only a few investigators have reported on the use of thermodilution cardiac output in awake rats. Therefore, we developed awake, freely-moving rat animal models for the measurement of ECG, arterial blood pressure, and thermodilution cardiac output.

MATERIALS AND METHODS

Male F344 (250-350 g) rats were utilized for the study. ECG and direct arterial blood pressure were measured in one set of animals (Model #1), while cardiac output via the thermodilution technique was measured in a second set of animals (Model #2). Anesthesia was induced for surgical preparation with sodium pentobarbital (40 mg/kg i.p.). The animals were prepared for surgery in a routine manner. A 1-cm skin incision was made with a #15 scalpel blade on the midline of the ventral cervical region. Blunt dissection was used to isolate the left common carotid artery and right external jugular vein.

Model No. 1: In a method similar to that described by Popovic and Popovic (7), polyethylene tubing (P.E. 50)¹ was used to cannulate both the left common carotid artery and right external jugular vein. The arterial catheter was inserted 3.5 to 4 cm so that its tip floated downstream in the descending portion of the aortic arch. The venous catheter was inserted 2.5 cm into the superior vena cava or right auricle. Catheters were secured to the vessel with 0000 surgical silk at three different locations, flushed with heparinized (4 units/ml) isotonic saline, and capped peripherally with a stainless steel plug. The catheters were routed subcutaneously with 17-gauge, stainless steel hypodermic tubing to the dorsal cervical area and exteriorized. Teflon-coated, single-stranded, stainless steel wires (34-gauge)² were then sutured intradermally through a small skin incision made with a #11 scalpel blade on the posterior aspect of all four extremities (Figure 1). The wires were likewise passed subcutaneously to the dorsal cervical region and exteriorized. The animals were then attached to a nylon jacket and spring tether³, placed in individual wire-bottom cages (12 x 12 in.), and allowed to recover for at least 24 to 48 hours before experimental manipulations were initiated.

Model No. 2: Rats were prepared for surgery as above and both the left carotid artery and right jugular vein were isolated. A thermocouple probe (0.5 mm O.D)⁴ was inserted into the left carotid artery and advanced 4 mm into the thoracic descending aorta, while P.E. 20 tubing⁵ was inserted 2.5 cm into the right auricle or superior vena cava via the right external jugular vein. The probe and P.E. tubing were exteriorized as stated above and the animals were attached to a nylon jacket and spring tether.

Phasic arterial blood pressure was measured with the carotid arterial catheter connected to a strain gauge transducer⁶ and transducer amplifier. The arterial catheter was intermittently connected to the transducer during a measurement period. Between measurement periods, the catheter was flushed with heparinized isotonic saline and capped with a stainless steel pin. Six-lead frontal plane electrocardiograms (ECG) were recorded from the implanted wire electrodes intermittently attached by alligator clamps to an ECG amplifier and chart recorder⁷. Interval and amplitude measurements were made under magnification with a vernier caliper. The same individual made the measurements on all ECG tracings. Cardiac output was measured in Model No. 2 by the thermodilution technique in which 150-200 μ l of room-temperature (20.5 - 22.0°C) normal saline was rapidly injected by a microliter syringe⁸, held in a clamp, through the venous catheter into the right auricle. Care was taken not to handle the syringe barrel. The aortic blood temperature was measured by the thermocouple probe, and the resulting thermodilution curve recorded by a chart recorder. Cardiac output was calculated by a commercially available cardiac output computer⁹. The average of two measurements taken 1 min apart was used as the cardiac output for a given time point. Values were normalized per 100 g of body weight. The injectate volume used in the calculation was corrected for the cannula dead space within the animal (8,9).

RESULTS

Arterial blood pressures and ECGs were evaluated as part of a toxicological study in a group of 49 F344 rats. Four hundred and thirty-five arterial blood pressure measurements were recorded, and 353 ECG tracings were analyzed before and serially after initiation of experimental treatment. Average values for the control measurements are reported and listed in Tables 1 and 2. Figure 2 demonstrates a representative 6-lead ECG tracing. Figure 3 shows a phasic blood pressure tracing with the simultaneously recorded Lead II ECG. Similarly, 197 measurements of cardiac output were accomplished before and serially after experimental treatment in a group of 27 rats. Control or baseline values are reported (Table 1), as well as a representative thermodilution curve (Figure 4). A subgroup of 15 control rats was monitored up to seven days after initiation of experimental procedures. In these rats, mean arterial blood pressures remained near baseline values; whereas, heart rates tended to decrease by the seventh day (Table 3). Functional success rate for the model was also evaluated over a 7-day period in a control and experimentally treated group. ECGs were determined to be successful if interpretable tracings could be obtained. Arterial blood pressure tracings were considered to be a success if a relatively undampened pulse pressure could be obtained for the accurate determination of mean arterial blood pressure. Arterial blood pressure determinations could readily be made through the 7-day period, while only 64% of the rats had functional ECGs at that time (Table 4). The most common causes for failure were breakage of the implanted wires and the development of 60-cycle interference.

DISCUSSION

The rat has been used extensively for cardiovascular studies in biomedical research. Many studies, however, have utilized anesthesia or various restraining devices which can alter hemodynamic function. With available instrumentation, ECG wires, catheters, and probes can be successfully implanted for measurement of a variety of cardiovascular indices in the awake state. Rats seem readily suited to the tether system described, as they are able to move freely and rarely disturb the jacket or tether. There appears to be minimal discomfort after instrumentation, as most animals return to food and water within 24 to 48 hours. The result is a relatively unstressed awake model, free from the influence of anesthesia and physical restraint.

Baseline values in this study agreed closely with values reported for cardiac output, mean arterial blood pressure, and heart rate in other awake models. Literature values for cardiac output are the most varied among the parameters studied and are dependent on the measurement technique, as well as the anesthetic state of the animal. Other investigators, utilizing thermodynamic dilution in conscious rats, have reported values ranging from 28.3 to 51.8 ml/min/100 g (2,8,9,10,11,12). Cardiac output in conscious rats ranges from 20.7 to 45.4 ml/min/100 g when measured by application of the Fick principle (1,13,14), dye dilution (15), electromagnetic flow probe (16), and reference sample microsphere method (12,17,18). From our own experience (unpublished observations) and from previously reported values (9,14,15), anesthesia generally causes cardiac output to be depressed as much as 30 to 40%.

Thermodilution cardiac output was utilized in this study because it did not require extensive surgical preparation (i.e., thoracotomy), it easily afforded the ability to make repeated measurements without the necessity for blood withdrawal, and it was inherently safe. Because of the difficulty involved in placing a thermocouple probe in the pulmonary artery of small animals, cardiac output must be measured transpulmonarily by the aortic thermodilution technique. This method tends to overestimate cardiac output due to the loss of thermal indicator in the pulmonary vascular bed (19). Nonetheless, this procedure has been shown to be accurate, reproducible, (11,19,20), as well as sensitive enough to detect changes following physiological or pharmacological interventions (9,12,21) in small animals like the rat.

Phasic arterial blood pressure and heart rate vary with the conscious state of the animal. Anesthetized rats generally have lowered arterial blood pressures and heart rates, but this response is not always clear-cut. Stability and normality of hemodynamic function are also difficult to maintain during prolonged anesthesia. In this study, individual animals demonstrated stable arterial blood pressures and heart rates when resting quietly in the cage. Grooming, drinking, and other natural behaviors, however, resulted in spontaneous excitation of heart rate and arterial pressure as previously reported (22). Therefore, to reflect basal levels, measurements were taken when the animals were calm.

There are many reports in the literature evaluating electrocardiographic alterations in the rat, but few have utilized an awake, relatively undisturbed model. In our hands, tracings adequate for measuring intervals and amplitudes were consistently achieved. The measured amplitudes and intervals agreed

closely with those previously published for the lightly anesthetized rat (23,24). This capability allowed monitoring of rate and rhythm, as well as the ability to observe subtle changes in the ECG pattern.

The uniqueness of the model lies in its ability to measure arterial blood pressure, as well as ECG, simultaneously. This, coupled with cardiac output, provided a much better overall cardiovascular profile than could be attained from arterial blood pressure and heart rate alone. However, the procedure utilized in this study did not permit the measurement of cardiac output and arterial blood pressure in the same individual. We have attempted femoral arterial cannulations for blood pressure measurements in rats also instrumented for the measurement of cardiac output, but have observed an unacceptable number of ischemic injuries in the rear leg distal to the catheter placement site. Although femoral arterial cannulations for chronically catheterized rats are becoming common in the literature, few investigators have reported this associated injury.

The techniques described have been successfully utilized in this laboratory to evaluate acute cardiovascular alterations in the rat without the uncertainty of anesthetic/drug interactions. Animals can be continuously monitored for several hours or intermittently connected to allow for measurements over several days. We have routinely used the model for continuous 6-hour arterial blood pressure monitoring and ECG evaluation. Repeated cardiac output determinations can be made at specified intervals. In our experimental investigations, cardiac output measurements have been made at 30-minute to hourly intervals through an 8-hour study period. Individual animals have been followed for up to one week during which cardiac output determinations were successfully made. However, a large group of rats have not been followed to determine long-term viability of the preparation. This

catheterized model has also been extensively utilized for intermittent micro blood sampling for arterial blood gas analysis. In summary, the tethered model allowed for repeated measurement of ECG and hemodynamic indices in the unanesthetized, relatively undisturbed state.

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FOOTNOTES

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2. Jersey Strand and Cable Inc., Washington, NJ
3. Alice King and Chatham, Los Angeles, CA
4. Bailey Instruments Inc., Saddlebrook, NJ
5. Clay Adams, Parsippany, NJ
6. Statham P23ID, Gould Inc., Oxnard, CA
7. Gould 2600, Gould Inc., Oxnard, CA
8. Hamilton Microliter #750, Hamilton Co., Reno, NV
9. Cardiotherm 500-AC-R, Columbus Instruments, Columbus, OH

TABLE 1

Baseline Arterial Blood Pressures and Cardiac Output
in Awake F344 Rats

	Mean	STD	Min	Max	N
Heart Rate, beats/min	386	27	330	450	49
Systolic blood pressure, mm Hg	147	10.4	128	170	49
Diastolic blood pressure, mm Hg	107	9.5	87	127	49
Mean blood pressure, mm Hg	121	9.5	102	141	49
Pulse pressure, mm Hg	40	3.9	31	52	49
Cardiac output, ml/min/100 g	36.4	4.9	30.2	50.4	27

Table 2**Baseline Lead II ECG in Awake Rats**

	Mean	STD	Min	Max	N
P-wave amplitude, mvolts	0.107	0.028	0.014	0.15	49
P-R interval, msec	40.38	0.32	35.00	45.00	49
QRS duration, msec	12.29	0.29	10.00	15.00	49
R wave amplitude, mvolts	0.879	0.186	0.550	1.30	49
Q-T interval, msec	74.59	5.48	57.40	84.80	49
T wave amplitude, mvolts	0.134	0.032	0.088	0.222	49
Mean QRS axis, degree	51.0	20.6	-15.0	90.0	49

Table 3
Arterial Blood Pressures and Heart Rates in Awake
F344 Rats over A Seven-Day Period^a

	Day 0	Day 1	Day 2	Day 3	Day 7
	n = 15	n = 15	n = 15	n = 15	n = 14
Heart Rate,	391	383	376	372	373
beats/min	±20	±39	±32	±35	±32
Systolic Blood	148	141	149	143	148
Pressure, mm Hg	±11	±14	±15	±13	±13
Diastolic Blood	107	102	108	102	109
Pressure, mm Hg	±10	±13	±13	±13	±12
Mean Blood	121	115	121	116	122
Pressure, mm Hg	±10	±13	±13	±12	±13
Pulse Pressure,	41	39	41	41	39
mm Hg	±4	±3	±4	±4	±4

^a Mean ± standard deviation

Table 4Success Rate Over Time of ECG and Arterial Blood Pressure Model^a

	Day 0	Day 1	Day 2	Day 3	Day 7
ECG	25/25 (100%)	24/25 (96%)	23/25 (92%)	21/25 (84%)	16/25 (64%)
Arterial Blood Pressure	25/25 (100%)	25/25 (100%)	25/25 (100%)	25/25 (100%)	24/25 (96%)

^a Values represent number of rats that had functional electrode wires and catheters.

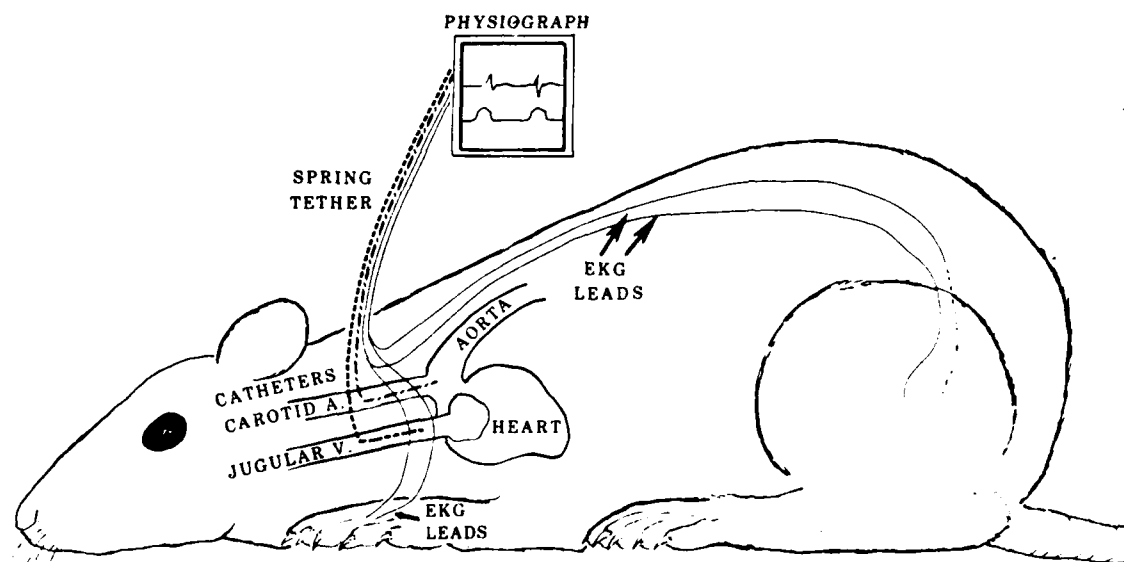
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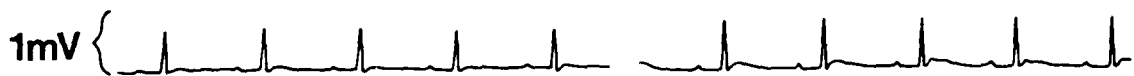
Figure 1. Schematic drawing of Model #1. The rat is instrumented for measurement of arterial blood pressure and simultaneous recording of electrocardiograms. Model #2 differs in having a thermocouple probe instead of a catheter in the carotid artery. Animals are housed individually in square, wire-bottom cages (12 x 12 in.). Catheters, wires, and/or probes are connected above the animal's cage to appropriate physiological monitoring equipment and chart recorders.

Figure 2. Representative 6-lead frontal plane electrocardiogram from an awake, tethered F344 rat. Paper speed = 100 mm/sec.

Figure 3. Lead II ECG and simultaneously recorded arterial blood pressure tracing from an awake, tethered F344 rat. Paper speed = 100 mm/sec.

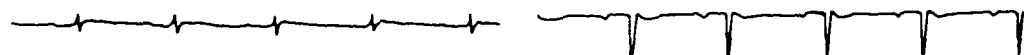
Figure 4. Thermodilution, cardiac output curve recorded from a thermocouple probe located in the aortic arch following a bolus injection of 150 μ l room-temperature isotonic saline into the right auricle.





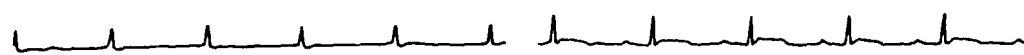
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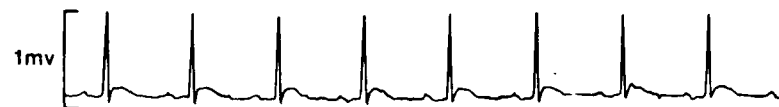
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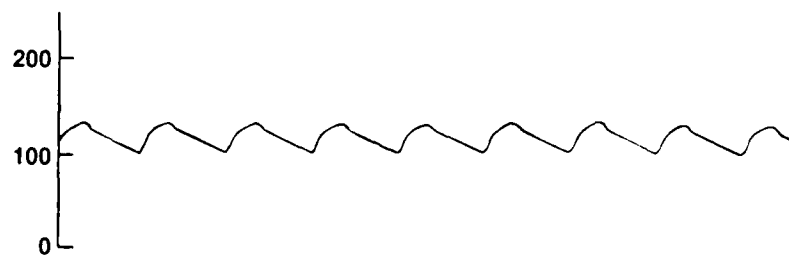
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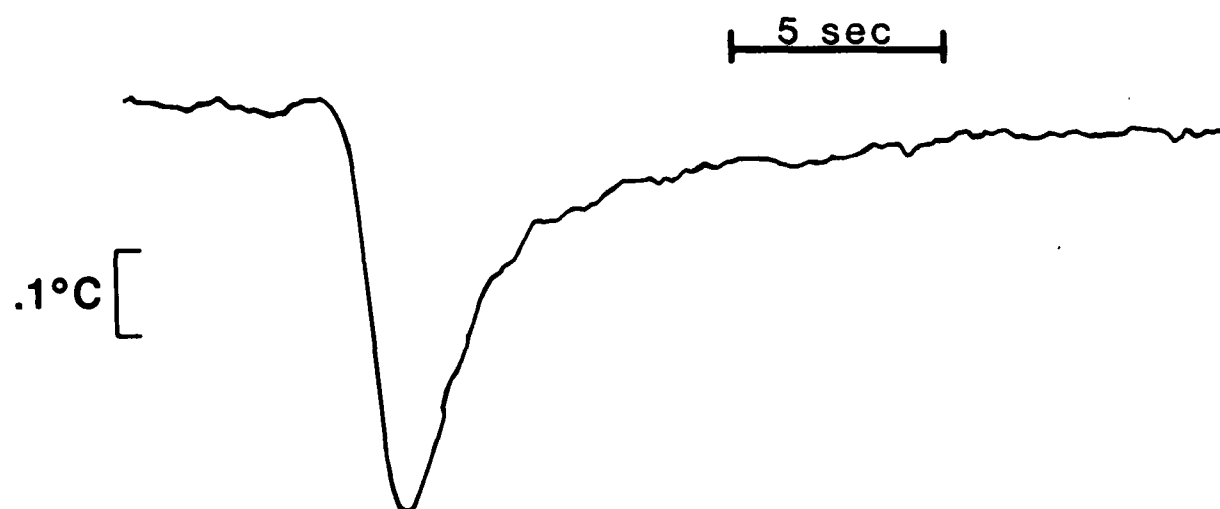
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Lead II ECG



Arterial Blood Pressure





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